

2AT

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
18 November 2004 (18.11.2004)

PCT

(10) International Publication Number
WO 2004/098521 A2

- (51) International Patent Classification⁷: **A61K** (74) Agent: TSAO, Rocky, Y.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).
- (21) International Application Number: PCT/US2004/013693 (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 28 April 2004 (28.04.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/466,599 30 April 2003 (30.04.2003) US (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (for all designated States except US): **TAIGEN BIOTECHNOLOGY** [CN/CN]; 7F, 138 Shin Ming Rd., Neihu Dist., Taipei 114 (CN).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **CHEN, Hua-Chien**; No. 129-13, Wolung Street, Taipei 106 (**). **SUN, Ying** [GB/GB]; 77 Links Road, London SW17 9EF (GB). **HUANG, Ying-Huey**; 62-91 Fu-Hsing Road, Fu-Hsing Village, Changhua 506 (**). **HSU, Ming-Chu** [US/US]; 929 East Essex Street, Glendora, CA 91740 (US). **LIN, Din-Lii** [US/US]; 100 West Orange Grove Avenue, Arcadia, CA 91006 (US).
- Published:**
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/098521 A2

(54) Title: TREATMENT AND DIAGNOSTICS OF CANCER

(57) Abstract: A method of determining whether a subject is suffering from or at risk for developing cancer. The method involves providing a sample from a subject, and determining the level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in the sample. The level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in the sample, if higher than that in a sample from a normal subject, indicates that the subject is suffering from or at risk for developing cancer. Also disclosed are a method of identifying a compound for treating cancer, a method of treating cancer, and a pharmaceutical composition or a packaged product for treating cancer.

5

TREATMENT AND DIAGNOSTICS OF CANCER

RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 60/466,599, filed April 30, 2003, the contents of which are incorporated herein by
10 reference.

BACKGROUND

G protein-coupled receptors (GPCRs) are the largest and most diverse family of transmembrane receptors. Responding to a wide range of stimuli including small
15 peptides, lipid analogs, amino-acid derivatives, and sensory stimuli such as light, taste, and odor, they transmit signals to the interior of the cell through interaction with heterotrimeric G proteins. It has been estimated that, of the 35,000 or so human genes, approximately 750 are GPCRs. About half of these sequences are likely to encode sensory receptors, leaving nearly 400 receptors that can be considered as potential targets
20 for drug development (Sautel and Milligan (2000) Curr Med Chem 7(9), 889-896; and Gurrath (2001) Curr Med Chem 8(13), 1605-1648). GPCRs are widely expressed and mediate most cell-cell communication in humans. Recent studies further highlight the expansive role that GPCRs play in promoting autocrine and paracrine influence on cellular transformation, tumor growth, invasion, and metastasis to distant organs (Ram
25 and Iyengar (2001) Oncogene 20(13), 1601-1606).

SUMMARY

This invention relates to use of GPCR genes as targets for treating cancer.

In one aspect, the invention features a method of determining whether a subject is suffering from or at risk for developing cancer (e.g., colon, liver, gastric, or prostate
30 cancer, or T cell leukemia). For example, the method includes providing a sample (e.g., a colon, liver, gastric, prostate, or blood sample) from a subject and determining the gene expression level of HM74, LGR6, GPR88, or GPR49 in the sample. If the gene

5 expression level of HM74, LGR6, GPR88, or GPR49 in the sample is higher than that in a sample from a normal subject, it indicates that the subject is suffering from or at risk for developing cancer. The gene expression level of HM74, LGR6, GPR88, or GPR49 can be determined by measuring the amount of the mRNA or the protein of HM74, LGR6, GPR88, or GPR49. The mRNA level can be determined, e.g., by in situ hybridization, 10 PCR, or Northern blot analysis. The protein level can be determined, e.g., by Western blot analysis. In another example, the method includes providing a sample from a subject and determining the protein activity level of HM74, LGR6, GPR88, or GPR49 in the sample. If the protein activity level of HM74, LGR6, GPR88, or GPR49 in the sample is higher than that in a sample from a normal subject, it indicates that the subject is 15 suffering from or at risk for developing cancer. The protein activity level of HM74, LGR6, GPR88, or GPR49 can be determined, e.g., by measuring GDP-GTP exchange on G-protein subunits following activation of HM74, LGR6, GPR88, or GPR49.

In another aspect, the invention features a method of identifying a compound for treating cancer. The method includes contacting a compound with a system (a cell 20 system or a cell-free system) containing an HM74, LGR6, GPR88, or GPR49 gene or an HM74, LGR6, GPR88, or GPR49 gene product, and determining the level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in the system. The level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in the presence of the compound, if lower than that in the absence of the compound, indicates that the 25 compound is a candidate for treating cancer. Such a compound can be any molecule, e.g., an anti-sense RNA, an antibody or its variant, or a non-peptidyl molecule.

Also within the scope of the invention is a method of treating cancer. The method includes identifying a subject suffering from or being at risk for developing cancer and administering to the subject a composition to decrease the level of HM74, LGR6, GPR88, 30 or GPR49 gene expression or protein activity in the subject.

The invention further features a pharmaceutical composition containing a pharmaceutically acceptable carrier and an effective amount of a compound. The compound, when administered to a subject in need thereof, decreases the level of HM74,

5 LGR6, GPR88, or GPR49 gene expression or protein activity in the subject. Thus, the pharmaceutical composition of the invention can be used for treating cancer.

Moreover, the invention features a packaged product including a container, an effective amount of a compound that decreases the level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in a subject, and a legend associated with the
10 container and indicating administration of the compound for treating cancer.

The details of one or more embodiments of the invention are set forth in the accompanying description below. Other features, objects, and advantages of the invention will be apparent from the detailed description, and from the claims.

DETAILED DESCRIPTION

15 This invention is based on the unexpected discovery that some GPCR genes are up-regulated in cancer cells. Accordingly, the invention provides methods for diagnosing and treating cancer by targeting these GPCR genes.

A diagnostic method of the invention involves comparing the gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 in a sample prepared from a
20 subject with that in a sample prepared from a normal subject, i.e., a subject who does not suffer from cancer. A higher gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 indicates that the subject is suffering from or at risk for developing cancer. For example, if the gene expression level in a test subject is 3-fold higher than that in a normal subject as determined by the method described in the examples below or
25 any analogous methods, the test subject is identified as being suffering from or at risk for developing cancer. The method of the invention can be used on its own or in conjunction with other procedures to diagnose cancer.

The gene expression level of HM74, LGR6, GPR88, or GPR49 can be determined at either the mRNA level or the protein level. Methods of measuring mRNA levels in a
30 tissue sample are known in the art. In order to measure mRNA levels, cells can be lysed and the levels of mRNA in the lysates or in RNA purified or semi-purified from the lysates can be determined by any of a variety of methods including, without limitation, hybridization assays using detectably labeled gene-specific DNA or RNA probes and

5 quantitative or semi-quantitative RT-PCR methodologies using appropriate gene-specific oligonucleotide primers. Alternatively, quantitative or semi-quantitative in situ hybridization assays can be carried out using, for example, tissue sections or unlysed cell suspensions, and detectably (e.g., fluorescently or enzyme) labeled DNA or RNA probes. Additional methods for quantifying mRNA include RNA protection assay (RPA) and
10 SAGE.

Methods of measuring protein levels in a tissue sample are also known in the art. Many such methods employ antibodies (e.g., monoclonal or polyclonal antibodies) that bind specifically to the target protein. In such assays, the antibody itself or a secondary antibody that binds to it can be detectably labeled. Alternatively, the antibody can be
15 conjugated with biotin, and detectably labeled avidin (a polypeptide that binds to biotin) can be used to detect the presence of the biotinylated antibody. Combinations of these approaches (including "multi-layer sandwich" assays) familiar to those in the art can be used to enhance the sensitivity of the methodologies. Some of these protein-measuring assays (e.g., ELISA or Western blot) can be applied to lysates of cells, and others (e.g.,
20 immunohistological methods or fluorescence flow cytometry) applied to histological sections or unlysed cell suspensions. Methods of measuring the amount of label depend on the nature of the label and are well known in the art. Appropriate labels include, without limitation, radionuclides (e.g., ^{125}I , ^{131}I , ^{35}S , ^3H , or ^{32}P), enzymes (e.g., alkaline phosphatase, horseradish peroxidase, luciferase, or β -galactosidase), fluorescent moieties
25 or proteins (e.g., fluorescein, rhodamine, phycoerythrin, GFP, or BFP), or luminescent moieties (e.g., QdotTM nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA). Other applicable assays include quantitative immunoprecipitation or complement fixation assays.

The protein activity level of HM74, LGR6, GPR88, or GPR49 can be determined,
30 e.g., by measuring GDP-GTP exchange on G-protein subunits following activation of HM74, LGR6, GPR88, or GPR49. See, e.g., Peltonen et al. (1998) Eur J Pharmacol 355, 275.

5 The invention also provides a method for identifying and manufacturing compounds (e.g., proteins, peptides, peptidomimetics, peptoids, antibodies, or small molecules) that decrease the gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 in a system. Compounds thus identified can be used, e.g., for treating cancer.

10 The candidate compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art. Such libraries include: peptide libraries, peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone that is resistant to enzymatic degradation);
15 spatially addressable parallel solid phase or solution phase libraries; synthetic libraries obtained by deconvolution or affinity chromatography selection; and the "one-bead one-compound" libraries. See, e.g., Zuckermann et al. (1994) *J Med Chem* 37, 2678-2685; and Lam (1997) *Anticancer Drug Des* 12, 145.

 Examples of methods for the synthesis of molecular libraries can be found in the art, for example, in: DeWitt et al. (1993) *PNAS USA* 90, 6909; Erb et al. (1994) *PNAS USA* 91, 11422; Zuckermann et al. (1994) *J Med Chem* 37, 2678; Cho et al. (1993) *Science* 261, 1303; Carrell et al. (1994) *Angew Chem Int Ed Engl* 33, 2059; Carell et al. (1994) *Angew Chem Int Ed Engl* 33, 2061; and Gallop et al. (1994) *J Med Chem* 37,1233. Methods of making monoclonal and polyclonal antibodies and fragments thereof are also known in the art. See, for example, Harlow and Lane, (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. The term "antibody"
25 includes intact molecules and fragments thereof, such as Fab, F(ab')₂, and Fv which are capable of binding to an epitopic determinant present in the HM74, LGR6, GPR88, or GPR49 protein.

 Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13, 412-421), or on beads (Lam (1991) *Nature* 354, 82-84), chips (Fodor (1993) *Nature* 364, 555-556), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent No. 5,223,409), plasmids (Cull et al. (1992) *PNAS USA* 89, 1865-1869), or phages (Scott and Smith (1990) *Science* 249, 386-390; Devlin (1990) *Science* 249, 404-406; Cwirla et

- 5 al. (1990) PNAS USA 87, 6378-6382; Felici (1991) J Mol Biol 222, 301-310; and U.S. Patent No. 5,223,409).

To identify compounds that decrease the gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 in a subject, a system containing the HM74, LGR6, GPR88, or GPR49 gene or an HM74, LGR6, GPR88, or GPR49 gene product (mRNA or
10 protein) is contacted with a candidate compound, and the gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 is evaluated relative to that in the absence of the candidate compound. In a cell system, the cell (e.g., a cancer cell) can be a cell that naturally expresses the HM74, LGR6, GPR88, or GPR49 gene, or a cell that is modified to express a recombinant HM74, LGR6, GPR88, or GPR49 gene, for example,
15 by having the HM74, LGR6, GPR88, or GPR49 gene fused to a heterologous promoter or by having the HM74, LGR6, GPR88, or GPR49 promoter fused to a heterologous gene. The gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 can be determined according to the methods described in the examples below, or any other methods well known in the art. If the gene expression or protein activity level of HM74,
20 LGR6, GPR88, or GPR49 is lower in the presence of the candidate compound than that in the absence of the candidate compound, the candidate compound is identified as being useful for treating cancer.

This invention further provides a method for treating cancer. Subjects to be treated can be identified, for example, by determining the gene expression or protein
25 activity level of HM74, LGR6, GPR88, or GPR49 in a sample prepared from a subject by methods described above. If the gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 is higher in the sample from the subject than that in a sample from a normal subject, the subject is a candidate for treatment with an effective amount of compound that decreases the gene expression or protein activity level of HM74,
30 LGR6, GPR88, or GPR49 in the subject. This method can be performed alone or in conjunction with other drugs or therapy.

The term "treating" is defined as administration of a composition to a subject, who has cancer, with the purpose to cure, alleviate, relieve, remedy, prevent, or

5 ameliorate the disorder, the symptom of the disorder, the disease state secondary to the disorder, or the predisposition toward the disorder. An "effective amount" is an amount of the composition that is capable of producing a medically desirable result, e.g., as described above, in a treated subject.

In one in vivo approach, a therapeutic composition (e.g., a composition containing
10 a compound identified as described above) is administered to the subject. Generally, the compound is suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected or implanted subcutaneously, intramuscularly, intrathecally, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. For
15 treatment of cancer, the compound can be delivered directly to the cancer tissue.

The dosage required depends on the choice of the route of administration; the nature of the formulation; the nature of the subject's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100 mg/kg. Wide
20 variations in the needed dosage are to be expected in view of the variety of compounds available and the different efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Encapsulation of the
25 compound in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Alternatively, a polynucleotide, such as one containing a nucleic acid sequence encoding an anti-sense HM74, LGR6, GPR88, or GPR49 RNA, can be delivered to the subject, for example, by the use of polymeric, biodegradable microparticle or
30 microcapsule delivery devices known in the art. Another way to achieve uptake of the nucleic acid is using liposomes, prepared by standard methods. The vectors can be incorporated alone into these delivery vehicles or co-incorporated with tissue-specific antibodies. Alternatively, one can prepare a molecular conjugate composed of a plasmid

5 or other vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells (Cristiano et al. (1995) J Mol Med 73, 479). Alternatively, tissue specific targeting can be achieved by the use of tissue-specific transcriptional regulatory elements (TRE) which are known in the art. Delivery of "naked DNA" (i.e., without a delivery vehicle) to an intramuscular,
10 intradermal, or subcutaneous site is another means to achieve in vivo expression.

The above-described polynucleotide can be an RNA interference agent, i.e., a duplex-containing RNA or a DNA sequence encoding it, which inhibits the expression of HM74, LGR6, GPR88, or GPR4 via RNA interference. RNA interference (RNAi) is a process in which double-stranded RNA (dsRNA) directs homologous sequence-specific
15 degradation of messenger RNA. In mammalian cells, RNAi can be triggered by 21-nucleotide duplexes of small interfering RNA (siRNA) without activating the host interferon response. As RNAi represses the expression of a specific gene, it can be used to treat a disease caused by abnormally high levels of expression of the gene. A duplex-containing RNA can be synthesized by techniques well known in the art. See, e.g.,
20 Caruthers et al., 1992, Methods in Enzymology 211, 3-19, Wincott et al., 1995, Nucleic Acids Res. 23, 2677-2684, Wincott et al., 1997, Methods Mol. Bio. 74, 59, Brennan et al., 1998, Biotechnol Bioeng., 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. It can also be transcribed from an expression vector and isolated using standard techniques.

In the above-mentioned polynucleotides (e.g., expression vectors), the nucleic
25 acid sequence encoding an RNAi agent or an anti-sense HM74, LGR6, GPR88, or GPR49 RNA is operatively linked to a promoter or enhancer-promoter combination. Enhancers provide expression specificity in terms of time, location, and level. Unlike a promoter, an enhancer can function when located at variable distances from the transcription initiation site, provided a promoter is present. An enhancer can also be
30 located downstream of the transcription initiation site.

Suitable expression vectors include plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses and adeno-associated viruses, among others.

5 Polynucleotides can be administered in a pharmaceutically acceptable carrier. As is well known in the medical art, the dosage for any one subject depends upon many factors, including the subject's weight, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for
10 administration of polynucleotide is about 10^6 to 10^{12} copies of the polynucleotide molecule. This dose can be repeatedly administered as needed. Routes of administration can be any of those listed above.

Also within the scope of the invention is a pharmaceutical composition that contains a pharmaceutically acceptable carrier and an effective amount of a compound
15 that decreases the gene expression or protein activity level of HM74, LGR6, GPR88, or GPR49 in a subject. The pharmaceutical composition can be used to treat cancer. The pharmaceutically acceptable carrier includes a solvent, a dispersion medium, a coating, an antibacterial and antifungal agent, and an isotonic and absorption delaying agent. The compound can also be packaged in a container with a label or an insert to indicate the
20 intended uses of the compound, i.e., treatment of cancer.

The compound of the invention can be formulated into dosage forms for different administration routes utilizing conventional methods. For example, it can be formulated in a capsule, a gel seal, or a tablet for oral administration. Capsules can contain any standard pharmaceutically acceptable materials such as gelatin or cellulose. Tablets can
25 be formulated in accordance with conventional procedures by compressing mixtures of the ligand with a solid carrier and a lubricant. Examples of solid carriers include starch and sugar bentonite. The compound can also be administered in a form of a hard shell tablet or a capsule containing a binder, e.g., lactose or mannitol, a conventional filler, and a tableting agent. The pharmaceutical composition can be administered via the parenteral
30 route. Examples of parenteral dosage forms include aqueous solutions, isotonic saline or 5% glucose of the active agent, or other well-known pharmaceutically acceptable excipient. Cyclodextrins, or other solubilizing agents well known to those familiar with the art, can be utilized as pharmaceutical excipients for delivery of the therapeutic agent.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/001418

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0158869	A	16-08-2001	
		AU 3495801 A	20-08-2001
		CA 2399791 A1	16-08-2001
		EP 1254115 A2	06-11-2002
		JP 2004502642 T	29-01-2004
		WO 0158869 A2	16-08-2001
		US 2002119972 A1	29-08-2002

THIS PAGE BLANK (USPTO)

5 The efficacy of a composition of the invention can be evaluated both in vitro and in vivo. For example, the composition can be tested for its ability to decrease the level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in vitro. For in vivo studies, the composition can be injected into an animal (e.g., an animal model) and its effects on cancer are then accessed. Based on the results, an appropriate dosage range and administration route can be determined.

10 The specific examples below are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications recited herein are hereby incorporated by reference in their entirety.

PROFILING OF LIVER TISSUES

Hepatoma tumor tissues

20 Primary HCCs and corresponding noncancerous liver tissues were obtained with informed consent from 40 patients who underwent hepatectomy. Patient profiles were obtained from medical records. Histopathological classification was performed according to the Edmondson grading system; clinical stages were determined according to the Union International Control Cancer TNM Classification. Histological analysis of paraffin embedded tissue was performed to verify the diagnoses. Tumor samples that were completely surrounded by malignant tissue were used in this study.

RNA extraction and cDNA preparation

30 RNA was extracted using an RNeasy kit (Qiagen, Valencia, California) according to manufacturer's instructions. RNA concentration was determined by spectrophotometry and adjusted to a concentration of 200 ng/ μ l. RNA (2 μ g) was reverse-transcribed using Superscript II enzyme (GIBCO BRL, Gaithersburg, MD) and 0.5 μ g oligo(dT)₁₂₋₁₆ (Amersham, Piscataway, NJ). The reaction mixture was incubated at 42°C for 50 min, followed by incubation at 72°C for 15 min. To ensure the fidelity of

- 5 mRNA extraction and reverse transcription, all samples were subjected to PCR amplification with oligonucleotide primers specific for the constitutively expressed gene GAPDH and normalization.

Quantitative RT-PCR

- The mRNA from each tissue sample was subjected to quantitative RT-PCR using
 10 140 primer pairs specifically designed for 140 non-olfactory GPCRs. Quantitative RT-PCR was performed on the LightCycler instrument using SYBR Green I dye. For each sample, the expression level of target GPCRs and the housekeeping gene (GAPDH) were determined. The ratio of GPCRs-to-GAPDH was calculated as the normalized value. All PCR reactions were performed using the LightCycler-FastStart DNA Master SYBR
 15 Green I kit (Roche). Cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 94°C for 5 sec, 57°C for 5 sec, and 72°C for 15 sec. Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized by ethidium bromide staining.

20

Table 1 **Primer sequences used for Quantitative RT-PCR**

25	GPR49	Forward primer: 5'-CACTGTCATTGCGAGC-3' Reverse primer: 5'-CGCAGGGATTGAAGGC-3'
	GPR88	Forward primer: 5'-CTGTACTGTAATGGTTGCT-3' Reverse primer: 5'-GTCTAACGGGTATCGCTT-3'
	HM74	Forward primer: 5'-ATAATAACCGCAGCACG-3' Reverse primer: 5'-AACCTTAGGCCGAGTC-3'
30	LGR6	Forward primer: 5'-GACCATCACCAACGGG-3' Reverse primer: 5'-CATGAGTCACACGGGA-3'
	GAPDH	Forward primer: 5'-TGAGCTGAACGGGAAG-3' Reverse primer: 5'-GTGTCGCTGTTGAAGT-3'

35

Results

5 Of the 140 GPCRs studied, 2 GPCRs (GPR49 and GPR88) were found to be up-regulated in the hepatoma cancer cells. The expression level of GPR49 showed at least 4- to 100-fold increase in approximately 38% of sample pairs. GPR49 was originally isolated as an orphan G protein-coupled receptor with leucine-rich repeat motifs in the N-terminal region (Hsu et al. (1998) *Mol Endocrinol* 12(12), 1830-1845). Although the
10 endogenous ligand as well as the biological functions of GPR49 has not yet been elucidated, overexpression of GPR49 mRNA was observed in 47% of hepatocellular carcinomas compared with corresponding noncancerous livers (Yamamoto et al. (2003) *Hepatology* 37(3), 528-533). GPR88 was originally cloned as a striatum-specific orphan GPCR with highest level of sequence homology to receptors for biogenic amines
15 (Mizushima et al. (2000) *Genomics* 69(3), 314-321). The expression pattern of GPR88 in human as well as in rodent was restricted in the striatum of brain tissue. It was found that some hepatocellular carcinoma samples showed marked up-regulation of GPR88. In contrast, noncancerous livers showed only low levels of GPR88. The average expression level in hepatocellular carcinoma was 18-fold higher than that in noncancerous liver.
20 Overexpression (tumor/normal ratio > 3) was found in 15 of 40 hepatocellular carcinomas (38%). The primer sequences of GPR49 and GPR88 used in quantitative RT-PCR reaction are listed in Table 1 above.

PROFILING OF COLON CANCER TISSUES

Colon tumor tissue

25 Fresh colorectal tissue samples were obtained from the cancerous and noncancerous parts of surgical specimens. Immediately after surgical removal, tissues were grossly dissected by a pathologist, snap frozen and stored in liquid nitrogen until analysis. Histological analysis of paraffin embedded tissue was performed to verify the diagnoses. Tumor samples that were completely surrounded by malignant tissue were
30 used in this study. For in-situ hybridization studies, all samples were immediately frozen and embedded in TissueTek OCT medium (Sakura, Tokyo, Japan) and stored at -80°C until further analysis.

Results

5 Of the 140 GPCRs investigated, 3 GPCRs (GPR49, HM74 and LGR6) were found to be up-regulated in the colon cancer tissue. Mean expression of GPR49 was 13-fold higher in the cancerous parts of the colon cancers. Overexpression (tumor/normal ratio > 3) was found in 32 of 40 colon cancers (80%). The expression level of HM74 was found to be elevated in 30% tissue pairs. Furthermore, elevated expression of LGR6 was also found in 15 of 40 colon cancer samples (38%). The primer sequences of GPR49, HM74, and LGR6 are listed in Table 1 above.

Table 2 Up-regulated expression of GPCRs in hepatoma or colon cancer

Gene name	GenBank	Regulation Status
	Accession Number	(Nos. of positive sample/total sample)
GPR49	NM_003667	Up in hepatoma (15/40)
		Up in colon cancer (32/40)
GPR88	NM_022049	Up in hepatoma (15/40)
HM74	NM_006018	Up in colon cancer (12/40)
LGR6	AK027377	Up in colon cancer (15/40)

EXPRESSION OF GPCRS IN TUMOR CELL LINES

Cell line information

20 To determine whether the elevated expression of GPCRs can be detected in cell lines of various origins, the expression levels of GPR49, GPR88, HM74, and LGR6 were examined in 23 human tumor cell lines. Cells were grown to 90% confluency, and total RNA was prepared using the RNeasy kit (Qiagen, Valencia, California) according to the manufacturer's instructions. Gene expression level was determined by quantitative RT-PCR using the primers listed in Table 1 above.

5 **Table 3 Cell lines used for GPCR profiling**

Cell name	Cell origin
MDA-MB-231	Human breast adenocarcinoma
MDA-MB-435	Human breast adenocarcinoma
MCF-7	Human breast carcinoma
DU4475	Human breast carcinoma, metastatic cutaneous nodule
DLD-1	Human colon adenocarcinoma
LoVo	Human colon adenocarcinoma
LS174T	Human colon adenocarcinoma
HT-29	Human colon adenocarcinoma
T-84	Human colon adenocarcinoma
SW403	Human colon adenocarcinoma
SW480	Human colon adenocarcinoma
WiDr	Human colon adenocarcinoma
AGS	Human gastric adenocarcinoma
NUGC	Human gastric cancer cell
HepG2	Human hepatocellular carcinoma
Huh-7	Human liver cancer
HH	Human cutaneous T cell leukemia / lymphoma
MOLT4	Human peripheral blood, acute T lymphoblastic leukemia
Jurkat J45.01	Human T lymphocyte, acute T cell leukemia
PC-3	Human prostate adenocarcinoma
DU145	Human prostate carcinoma
22RV1	Human prostate carcinoma
LNCaP	Human prostate carcinoma

Results

The expression levels of GPCRs in human cancer cell lines were normalized to the levels of GAPDH in individual samples. It was found that GPR49 was expressed in

10 high abundance in human hepatoma cell line HepG2 and Huh7 as well as in human colon

5 cancer cell line LoVo. Furthermore, gastric cancer AGS cells expressed highest level of GPR49. These results further confirmed the potential roles of GPR49 in tumor malignancy. In contrast, none of the breast cancer cell lines in this study (including MCF-7, MDA-MB-231, and MDA-MB-435) expressed significant levels of GPR49. HM74 was significantly expressed in several cancer cells, including hepatoma cells
10 HepG2 and Huh7, colon cancer cells WiDr, SW403, and HT-29, gastric cancer cells NUGC, prostate cancer cells 22RV1, and HH T cell leukemia. In contrast, expression of GPR88 was only found in HH cells. Expression of LGR6 was more restricted to colon cancer cell lines, including SW403, SW480, WiDr, T-84, LoVo, and DLD-1.

15 EXPRESSION OF GPR49 IN COLON CANCER TISSUE

The biopsy samples used to study gene expression in hepatoma and colon cancer contained mixed populations of normal and cancer cells. Therefore, in situ hybridization was used to examine the cellular localization of the GPCR of interest.

Tissue sections

20 Tumor samples were obtained from Chung-Gung Memorial Hospital. Tumor tissues were dissected and embedded in OCT (optimal cutting temperature) immediately after surgery. Tissue blocks were stored in -80°C refrigerator before sectioning. Sequential frozen sections ($10\text{ }\mu\text{m}$) were prepared using Leica CM1900 and thaw-mounted onto gelatin-coated slides. The slides were fixed with 4% paraformaldehyde for
25 10 minutes followed by 15% sucrose, and then air-dried overnight. The slides were covered with foil and stored at -80°C until hybridization. Tissue sections were stained with hematoxylin/eosin (H&E) for morphological examination.

Probe synthesis

30 DIG-labeled RNA probes were prepared using PCR amplification followed by in vitro transcription. Briefly, the selected regions of the target gene were amplified in PCR reactions, and the amplification products were verified by agarose gel electrophoresis. The DNA was then purified with phenol-chloroform extraction and resuspended in DEPC-treated water for storage at -20°C . RNA probes were then prepared using in vitro

5 transcription, and the labeling efficiency was determined by direct detection. Antisense RNA probes for GPR49 in situ hybridization are: forward primer 5'-GATCAGAATTGGAGTGTGGACCAT-3' and reverse primer 5'-TGTCGTGCAAAGCTGCCAAAAGTG-3'.

In situ hybridization

10 The frozen sections were thawed and washed with 2X SSC. Sections were then digested with proteinase K (1 µg/ml) for 30 minutes at 37°C followed by acetylation with 0.1 M triethanolamine-HCl. After prehybridization with hybridization buffer for 2 hrs at (Tm-25)°C, sections were hybridized with 50 µl DIG-labeled antisense RNA probe (5 ng/µl) for 18 hrs at the same temperature (hybridization with sense probes were included
15 as controls). Unhybridized single stranded RNA was then digested with RNase A (10 µg/ml RNase A in 10 mM Tris-HCl pH 8.0, 0.5 M NaCl, 1 mM EDTA) at 37°C for 30 minutes. After stringent washing procedures with SSC, signals were detected with alkaline phosphatase conjugated anti-DIG antibody (Roche, 500-fold dilution in 0.1 M Tris-HCl, 0.15 M NaCl, pH 8.0) and the substrate BCIP-NBT (Sigma). Sections were
20 incubated with anti-DIG antibody at RT for 4 hrs, and signals developed in BCIP-NBT at RT for 45 minutes to 1 hr. After counterstained with 0.2 % methylgreen, the sections were air-dried and mounted with Glycer-gel mounting media (Dako). The signals were examined under microscopy (Olympus BX 40) and recorded using digital camera (Olympus C-4040).

25 Results

It was found that the mRNA level of GPR49 was markedly higher in most of the colon cancers. In order to further confirm these findings, additional studies were performed to determine the histological distribution GPR49 mRNA in specimens from cancerous parts of colon cancer and the corresponding normal colon mucosa using in situ
30 hybridization. High abundance of GPR49 transcript was specifically detected in transformed epithelial cells but not in normal mucosa cells. In order to demonstrate the specificity of the probe, specimens derived from cancerous parts were hybridized with sense and antisense probes of GPR49. Only the antisense probe produced strong signal.

5 in cancer specimens. The preferential localization of GPR49 in cancer cells suggests that GPR49 is a useful diagnostic marker as well as a potential therapeutic target.

Further, GPR49 was stably expressed in a human colon cancer cell line (SW480), followed by growth experiments both *in vitro* and *in vivo*. It was found that increased GPR49 expression promoted tumor growth, indicating that GPR49 can be used as a
10 diagnostic marker and a therapeutic target of cancer.

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless
15 expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to
20 various usages and conditions. Thus, other embodiments are also within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of determining whether a subject is suffering from or at risk for developing cancer, the method comprising:
providing a sample from a subject; and
determining a level of HM74 or LGR6 gene expression or protein activity in the sample,
wherein the level of HM74 or LGR6 gene expression or protein activity in the sample, if higher than that in a sample from a normal subject, indicates that the subject is suffering from or at risk for developing cancer.
2. The method of claim 1, wherein the cancer is colon, liver, gastric, or prostate cancer, or T cell leukemia.
3. A method of determining whether a subject is suffering from or at risk for developing cancer, the method comprising:
providing a sample from a subject; and
determining a level of GPR88 gene expression or protein activity in the sample,
wherein the level of GPR88 gene expression or protein activity in the sample, if higher than that in a sample from a normal subject, indicates that the subject is suffering from or at risk for developing cancer.
4. The method of claim 3, wherein the cancer is liver cancer or T cell leukemia.
5. A method of determining whether a subject is suffering from or at risk for developing colon or gastric cancer, the method comprising:
providing a sample from a subject; and
determining a level of GPR49 gene expression or protein activity in the sample,

wherein the level of GPR49 gene expression or protein activity in the sample, if higher than that in a sample from a normal subject, indicates that the subject is suffering from or at risk for developing colon or gastric cancer.

6. A method of identifying a compound for treating cancer, the method comprising:

contacting a compound with a system containing an HM74 or LGR6 gene or an HM74 or LGR6 gene product; and

determining a level of HM74 or LGR6 gene expression or protein activity in the system,

wherein the level of HM74 or LGR6 gene expression or protein activity in the presence of the compound, if lower than that in the absence of the compound, indicates that the compound is a candidate for treating cancer.

7. The method of claim 6, wherein the cancer is colon, liver, gastric, or prostate cancer, or T cell leukemia.

8. A method of identifying a compound for treating cancer, the method comprising:

contacting a compound with a system containing a GPR88 gene or a GPR88 gene product; and

determining a level of GPR88 gene expression or protein activity in the system, wherein the level of GPR88 gene expression or protein activity in the presence of the compound, if lower than that in the absence of the compound, indicates that the compound is a candidate for treating cancer.

9. The method of claim 8, wherein the cancer is liver cancer or T cell leukemia.

10. A method of identifying a compound for treating colon or gastric cancer, the method comprising:
 - contacting a compound with a system containing a GPR49 gene or a GPR49 gene product; and
 - determining a level of GPR49 gene expression or protein activity in the system, wherein the level of GPR49 gene expression or protein activity in the presence of the compound, if lower than that in the absence of the compound, indicates that the compound is a candidate for treating colon or gastric cancer.
11. The method of claim 10, wherein the compound is an antibody.
12. The method of claim 11, wherein the antibody is a monoclonal antibody.
13. A method of treating cancer, the method comprising:
 - identifying a subject suffering from or being at risk for developing cancer; and
 - administering to the subject a composition to decrease a level of HM74 or LGR6 gene expression or protein activity in the subject.
14. The method of claim 13, wherein the cancer is colon, liver, gastric, or prostate cancer, or T cell leukemia.
15. A method of treating cancer, the method comprising:
 - identifying a subject suffering from or being at risk for developing cancer; and
 - administering to the subject a composition to decrease a level of GPR88 gene expression or protein activity in the subject.
16. The method of claim 15, wherein the cancer is liver cancer or T cell leukemia.
17. A method of treating colon or gastric cancer, the method comprising:

identifying a subject suffering from or being at risk for developing colon or gastric cancer; and

administering to the subject a composition to decrease a level of GPR49 gene expression or protein activity in the subject.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a compound that decreases a level of HM74 or LGR6 gene expression or protein activity in a subject.

19. The composition of claim 18, wherein the subject suffers from or is at risk for developing cancer.

20. The composition of claim 19, wherein the subject suffers from or is at risk for developing colon, liver, gastric, or prostate cancer, or T cell leukemia.

21. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a compound that decreases a level of GPR88 gene expression or protein activity in a subject.

22. The composition of claim 21, wherein the subject suffers from or is at risk for developing cancer.

23. The composition of claim 22, wherein the subject suffers from or is at risk for developing liver cancer or T cell leukemia.

24. A packaged product comprising:
a container;
an effective amount of a compound that decreases a level of GPR49 gene expression or protein activity in a subject; and

a legend associated with the container and indicating administration of the compound for treating colon or gastric cancer.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
18 November 2004 (18.11.2004)

PCT

(10) International Publication Number
WO 2004/098521 A3

- (51) International Patent Classification⁷: **C12Q 1/68**, C07H 21/02, 21/04
- (21) International Application Number: **PCT/US2004/013693**
- (22) International Filing Date: 28 April 2004 (28.04.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/466,599 30 April 2003 (30.04.2003) US
- (71) Applicant (for all designated States except US): **TAIGEN BIOTECHNOLOGY [CN/CN]**; 7F, 138 Shin Ming Rd., Neihu Dist., Taipei 114 (CN).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **CHEN, Hua-Chien**; No. 129-13, Wolung Street, Taipei 106 (**). **SUN, Ying** [GB/GB]; 77 Links Road, London SW17 9EF (GB). **HUANG, Ying-Huey**; 62-91 Fu-Hsing Road, Fu-Hsing Village, Changhua 506 (**). **HSU, Ming-Chu** [US/US]; 929 East Essex Street, Glendora, CA 91740 (US). **LIN, Din-Lii** [US/US]; 100 West Orange Grove Avenue, Arcadia, CA 91006 (US).
- (74) Agent: **TSAO, Rocky, Y.**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:
23 June 2005
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TREATMENT AND DIAGNOSTICS OF CANCER

(57) Abstract: A method of determining whether a subject is suffering from or at risk for developing cancer. The method involves providing a sample from a subject, and determining the level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in the sample. The level of HM74, LGR6, GPR88, or GPR49 gene expression or protein activity in the sample, if higher than that in a sample from a normal subject, indicates that the subject is suffering from or at risk for developing cancer. Also disclosed are a method of identifying a compound for treating cancer, a method of treating cancer, and a pharmaceutical composition or a packaged product for treating cancer.

WO 2004/098521 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/13693

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : C12Q 1/68; C07H 21/02, 21/04 US CL : 435/6; 536/23.5, 24.31, 24.33 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/6; 536/23.5, 24.31, 24.33 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/084298 A2 (GLAXO GROUP LIMITED) 24 October 2002 (24.10.2004), especially pages 4-6	1 and 2
A	US 5,994,076 A (CHENCHIK et al) 30 November 1999 (30.11.1999), especially columns 12-13 and 35.	1 and 2
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 23 March 2005 (23.03.2005)		Date of mailing of the international search report 27 APR 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230		Authorized officer <i>Carla Myers</i> Carla Myers Telephone No. 571-272-1600

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/13693

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
 2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
 4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1 and 2, with respect to HM74
- Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/13693

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search/examination fees must be paid.

Group I, claims 1 and 2 (in part), drawn to methods for diagnosing cancer by detecting HM74.

Group II, claims 1 and 2 (in part), drawn to methods for diagnosing cancer by detecting LGR6.

Group III, claims 3 and 4, drawn to methods for diagnosing cancer by detecting GPR88.

Group IV, claim 5, drawn to methods for diagnosing cancer by detecting GPR49.

Group V, claims 6 and 7 (in part), drawn to methods for screening for compounds that modulate HM74 expression or activity.

Group VI, claims 6 and 7 (in part), drawn to methods for screening for compounds that modulate LGR6 expression or activity.

Group VII, claims 8 and 9, drawn to methods for screening for compounds that modulate GPR88 expression or activity.

Group VIII, claims 10-12, drawn to methods for screening for compounds that modulate GPR49 expression or activity.

Group IX, claims 13-14 (in part), drawn to methods of treating cancer using a compound that targets HM74.

Group X, claims 13-14 (in part), drawn to methods of treating cancer using a compound that targets LGR6.

Group XI, claims 15-16, drawn to methods of treating cancer using a compound that targets GPR88.

Group XII, claim 17, drawn to methods of treating cancer using a compound that targets GPR49.

Group XIII, claims 18-20 (in part), drawn to a pharmaceutical that decreases expression of HM74.

Group XIV, claims 18-20 (in part), drawn to a pharmaceutical that decreases expression of LGR6.

Group XV, claims 21-23, drawn to a pharmaceutical that decreases expression of GPR88.

Group XVI, claim 24, drawn to a package with a composition that decreases expression of GPR49.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/13693

The inventions listed as Groups I-XVI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The feature linking the recited inventions are the nucleic acid and proteins of HM74, LGR6, GPR88 and GPR49. However, as set forth on page 13 of the disclosure, the nucleic acids and proteins of HM74, LGR6, GPR88 and GPR49 were known in the prior art at the time the invention was made and are specifically disclosed in GenBank Accession Nos. NM_006018, AK027377, NM_022049 and NM_003667. Further, as set forth in the disclosure at page 12, the prior art of Yamamoto (see citation to Hepatology (2003) 37:528-533)) teaches that expression of GPR49 is associated with the occurrence of hepatocellular carcinoma. As the feature of nucleic acids and proteins of HM74, LGR6, GPR88 and GPR49 do not represent a contribution over the prior art, the claims lack a special technical feature that is the same as or that corresponds to a special technical feature of the other claimed inventions. Thus, there is no special technical feature linking the recited Groups, as would be necessary to fulfill the requirement for unity of invention.

It is noted that the claims of Groups I, II, V, VI, IX, X, XIII and XIV are presented in improper Markush format, as distinct products and distinct methods are improperly joined in the claims. The claims encompass methods that require the use of HM74 or LGR6 and pharmaceuticals that decrease the expression of HM74 or LGR6. HM74 and LGR6 represent distinct chemical compounds that differ in their structure, physio-chemical properties and biological activities. HM74 and LGR6 have different nucleotide and amino acid sequences, have different functional properties, bind to distinct target nucleic acid and protein sequences and have different melting temperatures (for the nucleic acids) and isoelectric points (for the proteins). As the

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/13693

products and methods encompassed by the claims do not share a special technical feature, the distinct products and methods may not properly be presented in the alternative. Accordingly, the claims have been separated into a number of groups corresponding to the number of different inventions encompassed by the claims, and the claims will be searched only as they read upon the invention of the elected group. For the same reasons, the remainder of the claims have been separated in a number of groups corresponding to the number of different inventions encompassed thereby. Similarly, the nucleic acids and proteins of GPR88 and GPR49 represent distinct chemical compounds that also differ with respect to their nucleotide and amino acid sequence, their biological activities and effects, and their binding activities. Given the differences in the structure, function and effect of HM74, LGR6, GPR88, and GPR49, these compounds are not considered to share a special technical feature as would be necessary to fulfill the requirement for unity of invention. These distinct compounds do not have both a "common property or activity" and a common structure as would be required to show that the inventions are "of a similar nature".

Continuation of B. FIELDS SEARCHED Item 3:

DIALOG: MEDLINE, CA, BIOSIS, EMBASE; WEST: US, EP, JP, WO Patents

search terms: HM74, GPR109B, G protein coupled receptor 109B, PUMaG, puma-g, cancer, tumor, hepatocellular, colon, prostate, leukemia, liver, gastric, hepatocarcinoma, colorectal

THIS PAGE BLANK (USPTO)